MUSCULAR MECHANICAL RECOVERY WITH LASER

GIOVANA LEITÃO SENE, ANTÔNIO CARLOS SHIMANO, CELSO HERMÍNIO FERRAZ PICADO

ABSTRACT

Objective: We evaluated the influence of low-power laser irradiation, either associated or not with immobilization, on the mechanical properties of the gastrocnemius muscle submitted to experimental injury with an impact mechanism. Methods: Seventy female Wistar rats were divided into 7 experimental groups: group 1 – control; group 2 – injury; group 3 – injury-laser irradiation; group 4 – injury-immobilization for 24-hour laser irradiation; group 5 - injury-immobilization for 72-hour laser irradiation; group 6 – injury-immobilization for 24 hours; group 7 – injury-immobilization for 72 hours. The IBRAMED® Laser-pulse equipment (670 nm) was used for biostimulation. The muscles were submitted to mechanical assays in an EMIC® universal testing machine and load x stretching graphs were used to calculate the mechanical properties, i.e., at the proportionality limit and maximum limit. Results: The injury provoked a reduction of load at the proportionality limit and at the maximum limit compared to group 1 ($p < 0.05$). Groups 2, 4, 5 and 7 differed significantly from group 1. The property of stretching at the proportionality limit differed significantly between group 1 and groups 4, 5, 6 and 7 and between groups 2 and 4. The property of stretching at the maximum limit differed between groups 1 and 5 and between groups 3 and 5. Conclusion: The association of immobilization for 24 and 72 hours with laser therapy did not improve the mechanical properties of the muscle, whereas the separate use of each treatment modality was more effective.

Keywords: *Laser therapy. Low-Level. Immobilization. Biomechanics.*

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INTRODUCTION

Muscular trauma represent a large number of injuries in professional and recreational sports activities [1,2] and may occur by several mechanisms.[3]

The biological processes occurring after a muscular injury are: inflammation, repair and remodeling, and these events are interconnected and time-dependent.[2,4,5]

Immobilization usually is the method of choice for treating injuries of the musculoskeletal system, although it may lead to structural changes such as atrophy, increased connective tissue, fibrosis, extension ability and muscular resistance loss[6-8] in addition to synthesis disorders.[9,10] Other therapeutic modalities such as a combination of immobilization, ice, compression and lifting (RICE)[4], ultra-sound and laser are also employed.

Early mobilization has been preferred over immobilization, because it leads to a faster and more intense revascularization on the injury area with resultant muscular fibers regeneration.[4]

The optimal treatment approach for muscular injuries has not yet been established, with different therapies using physical or chemical means in place.

The effect of laser irradiation (AsGa) was evaluated on muscular repair after lesions caused by myotoxins and evidencing that the doses employed were ineffective to promote significant results compared to morphological changes.[11]

However, using a similar methodology to the laser-irradiation therapy (HeNe), concerning cicatritial and regenerative processes, effective histological results were seen, consisting on the presence of differentiating cells with centralized nucleus.[12]

Our purpose was to assess the influence of low-power laser irradiation, either associated to immobilization or not, on the recovery of mechanical properties of the gastrocnemius muscle submitted to experimental injury by mechanism of impact.

MATERIALS AND METHODS

Seventy female *Rattus Norvegicus Albinus* Wistar strain rats were used, with body mass ranging from 160 to 260 g (mean: 215.34±12.67). Upon approval of the Committee of Ethics in Animal Experimentation- CEUA (04.1.663.53-6), these animals were kept in collective containment cages in groups of three animals, at room temperature receiving water and standard ration *ad libitum*. The animals were randomly divided into 7 experimental groups composed by 10 animals each. Group 1 (Control) was kept under basal conditions for 8 days; Group 2 (Injury) submitted to experimental muscular injury and not submitted to any kind of treatment; Group 3 (Injury Laser), submitted to muscular injury and irradiated with laser for 8 consecutive days; Group 4 (Injury Immobilization 24h Laser), submitted to muscular injury, immobilized for 24 hours and laser-irradiated for 8 consecutive days; Group 5 (Injury Immobilization 72 h Laser), submitted to muscular injury, immobilized for 72 hours and laser-irradiated for 8 consecutive days; Group 6 (Injury Immobilization 24h), submitted to muscular injury and im-

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Medical School., Ribeirão Preto campus, and Institute of Chemistry, São Carlos – USP.
Department of Locomotive Apparatus Biomechanics, Rehabilitation and Medicine USP Medical School, Ribeirão Preto campus
Correspondences to: Departamento de Biomecânica, Medicina e Reabilitação do Aparelho Locomotor da Faculdade de Medicina de Ribeirão Preto da USP.Avenida Bandeirantes, 3900 - 11o andar- Ribeirão Preto/SP - Brasil – CEP:14048-900. E-mail: *giovana_leitao@yahoo.com.br*

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mobilized for 24 hours, and; Group 7 (Injury Immobilization 72h), submitted to muscular injury and immobilized for 72 hours.

For provoking an experimental injury by mechanism of impact, similar device to another one proposed by other authors[13,14] was built, which consists of two adjustable telescopic metal shafts, weighting 200 g and designed with a plastic support. Between the shafts, a guide was designed in clear acrylic material for guiding the load during free fall.

This device enables the release of a 200g load from a 30-cm height to reach the muscular venter of gastrocnemius with coxofemoral and knee joints extended and the ankle flexed at 90°, a similar protocol as used by some authors.[14] (Figures 1 and 2)

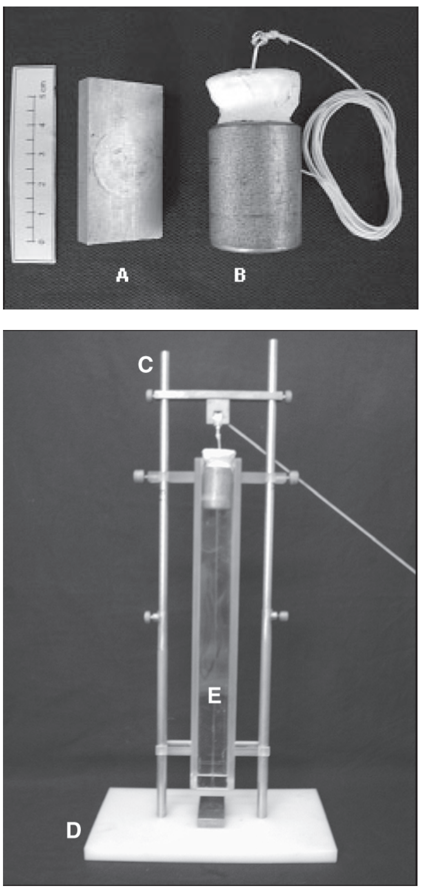

Figure 1 – *Device developed for producing an experimental injury. (A) Oblique view of the metal support surface of animal's gastrocnemius muscle (B) 200g load with guiding wire. Figure 2(C) Adjustable telescopic metal shafts (B) Plastic support (E) Clear acrylic guide.*

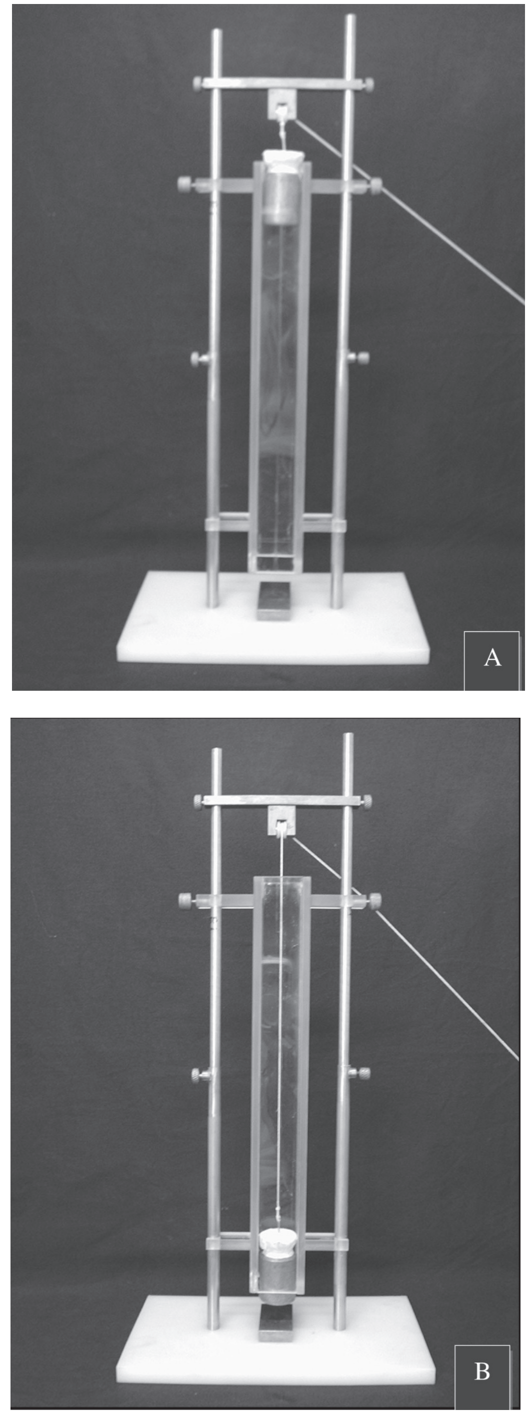

Figure 2(A) – *Device developed for producing an experimental injury. Frontal view with load at initial position. 2(B) Frontal view with load at final position under the contact surface.*

The animals were previously anesthetized with ketamine hydrochloride (60 mg/kg) and Xylazin (15 mg/kg) and the injury area on right paw was trichotomized, i.e., at the muscular gastrocnemius venter.

The equipment employed to irradiate the muscular injury area was the Laserpulse (IBRAMED®), with a laser probe able to irradiate a 670-nm wavelength. Equipment's features such as power and irradiation time were verified by means of a device called Laser Power/Energy Monitor calibrated by NIST (National Institute Standards and Technology) according to ISO 10012-1 rules, for confirming the parameters of the equipment employed.

Animals from groups 4, 5, 6 and 7 were submitted to plastered immobilization after experimental trauma, which included pelvis, hip, knee and ankle.

In order to allow animals of groups 4 and 5 to be submitted to treatment by laser irradiation, a window was opened on the plastered cast for fitting the laser probe.

The animals were sacrificed according to a protocol established for each group, by anesthetic overdose of sodium thiopental. Gastrocnemius muscles of the right paws were dissected and immediately submitted to traction mechanical assay. Dissection implied on the removal of all soft parts of the animal, keeping only the gastrocnemius muscle proximally inserted into the femur and distally into calcaneus.

Mechanical Assay

Those muscles were submitted to traction mechanical assay and they were fixated on the assay machine by means of a device attaching, on one side the femoral shaft and on the other, the paw, enabling muscular stretching.

An EMIC brand universal assay machine, model DL 10.000 was employed. The values for load and stretching for each assay were recorded on a computer attached to the machine and, with the aid of a software, the graph load vs. stretching was built, through which the mechanical properties of load and stretching at proportionality limit were obtained.

Data provided by the mechanical assay were assessed by means of Instat v. 3.00 by *Graphpad Software*. The simultaneous analysis of the groups was performed by applying the ANOVA test and, for comparison purposes between groups, the Tukey-Kramer test, both with a significance level of 5% ($p<0.05$).

RESULTS

Load at Proportionality Limit (LPL)

The mean value for load at proportionality limit (LPL) was: group 1- Control (30.63±3.23) N; group 2- Injury (25.87±2.92) N; group 3- Injury Laser (28.21±2.99) N; group 4- Injury Immobilization 24h Laser (24.51±4.08) N; group 5- Injury Immobilization 72h Laser (21.76±3.30) N; group 6- Injury Immobilization 24h (30.48±2.76) N; and group 7- Injury Immobilization 72h (24.56±2.06) N.

The simultaneous analysis of groups (ANOVA) showed a statistical difference ($p< 0.0001$). By means of the Tukey-Kramer test we could find a significant difference between the control group and the groups Injury, Injury Immobilization 24 h Laser, Injury Immobilization 72 h Laser and Injury Immobilization 72 h ($p<0.001$). The group Injury Immobilization 24h showed a significant difference when compared to group Injury ($p<0.01$), showing stronger load support at the proportionality limit.

Stretching at Proportionality Limit

The mean value for stretching at proportionality limit (SPL) was: Group 1- Control (10.40±3.05) mm; group 2- Injury (9.28±1.83) mm; group 3- Injury Laser (10.14±3.88) mm; group 4- Injury Immobilization 24h Laser (7.33±1.18) mm, group 5- Injury Immobilization 72h Laser (6.36±1.75) mm, group 6- Injury Immobilization 24h (8.08±1.99) mm and group 7- Injury Immobilization 72h (II72) of (7.62±1.66) mm.

The simultaneous analysis of the groups (ANOVA) showed a statistical difference ($p=0.0032$). By means of the Tukey-Kramer test we could find a significant difference between Control Group and Injury Immobilization 72h Laser group ($p<0.05$) indicating that group II72L stretched less upon the applied load. The other groups did not show statistical differences compared to control group and among each other.

Load at Maximum Limit (LML)

The mean value for load at maximum limit (LML) was: group 1- Control (35.43±2.46) N; group 2- Injury (28.05±3.25) N; group 3- Injury Laser (31.33±2.74) N; group 4- Injury Immobilization 24h Laser (27.48±3.71) N; group 5- Injury Immobilization 72h Laser (24.32±3.99) N; group 6- Injury Immobilization 24h (34.25±3.02) N; e group 7- Injury Immobilization 72h (28.23±2.10) N.

The simultaneous analysis of the groups (ANOVA) showed a statistical difference ($p< 0.001$). By means of the Tukey-Kramer test we could find a significant difference between Control Group and the groups Injury, Injury Immobilization 24h Laser, Injury Immobilization 72h Laser and Injury Immobilization 72h ($p<0.001$), with control group showing superior values when compared to those groups. In the comparison between groups Injury and Injury Immobilization 24h a statistical difference was found ($p<0.001$), with group II24h presenting statistically similar mean values to control group; between groups Injury Laser and Injury Immobilization 72h Laser ($p<0.001$) with group IL presenting statistically similar mean values to control group.

Stretching at Maximum Limit (SML)

The mean value for stretching at maximum limit (SML) was: group 1- Control (14.65±2.54) mm; group 2- Injury (11.67±1.28) mm; group 3- Injury Laser (13.07±3.56) mm; group 4- Injury Immobilization 24h Laser (10.02±2.35) mm; group 5- Injury Immobilization 72h Laser (8.48±1.62) mm; group 6- Injury Immobilization 24h (11.31±1.86) mm; e group 7- Injury Immobilization 72h (10.31±1.90) mm.

The simultaneous analysis of the groups (ANOVA) showed a statistical difference ($p< 0.0001$). By means of the Tukey-Kramer test we could find a significant difference between Control Group and the groups Injury Immobilization 24h Laser, Injury Immobilization 72h Laser, Injury Immobilization 72h ($p<0.001$), results statistically inferior to control group; and between Control group and Injury Immobilization 24 h group ($p<0.05$). For this property, the group Injury Laser showed similar statistical results as control group.

DISCUSSION

Several animals have been used in experimental studies. In this particular study, we chose rats for being easy to handle, low-cost, anesthesia-resistant animals with a similar musculoskeletal structure as the human.[15,16]

The selected muscle was gastrocnemius, for its location and function. This muscle works under extreme physical activity conditions, increasing the risk of injuries and ruptures[17] in addition to show high injury rates during sports practice.[18]

Muscular contusion by direct trauma is an injury that usually occurs during sports practice. The reproduction of this kind of trauma was enabled by the use of an experimental muscle injury induction device, which promoted a non-invasive direct impact.

In our study, we used the Laser biostimulation starting 2 hours after a muscular injury was produced[12], but the experimental period was adjusted to 8 days with a 3 J/cm^2 dosage. This period in which the animals were submitted to different therapies was selected because we chose to evaluate the mechanical behavior of the injured muscle under the influence of laser irradiation at acute phase. The dosage was 3 J/cm^2 because it causes analgesic, anti-inflammatory and regenerative effects.[19]

The rupture site on tensioned tested muscles was the muscle venter, i.e., at injury site for 95% of the mechanical assays. Experimentally, it was evidenced that, after 10 days of trauma, tensioned tested muscles showed rupture on the intact portion of the muscle, suggesting that cicatritial tissue's resistance to stretching became stronger than that of the muscular tissue at that point.[4]

The groups Injury Laser and Injury Immobilization 24h did not show significant difference compared to control group for the property load at proportionality limit, suggesting that the employed experimental therapies were effective for repairing.
Concerning the property stretching at proportionality limit, a statistical difference was found between Control and Injury Immobilization 72h Laser groups, suggesting that the association of laser with immobilization for 72 hours did not cause improvement to the property. Between groups Injury Laser and Injury Immobilization 72 h Laser, a statistical difference was found, suggesting that the use of laser alone promoted an improvement to the property.
When the mean values for load at maximum limit are compared, a statistical difference is seen between control group and groups Injury, Injury Immobilization 24 h Laser, Injury Immobilization 72h Laser and Injury Immobilization 72h, suggesting that the groups injury and injured and irradiated can afford a lighter load compared to control group. Therefore, the association of laser therapy with immobilization did not promote any improvement to this mechanical property.
No statistical difference was found for load at maximum limit between control group and groups Injury Laser and Injury Immobilization 24h; however, there was a drop of 11.57 and 3.33%, respectively, on numeric values. These results suggest that the use of each therapeutic modality alone was more effective in improving the property.
The stretching at maximum limit property for Control and Injury Laser groups showed statistically superior mean values to injury group. There was a percent increase of 25.6% and 14.5% respectively in the comparison of these groups, indicating that the Injury group stretched less upon the load applied, showing different mechanical behavior curves compared to control group.
Control group, when compared to the others, showed a significant difference, which means that the groups treated with a combination of immobilization and laser or with immobilization alone assumed statistically lower values for stretching at maximum limit compared to control group.
Laser is a therapeutic resource that evidenced histological changes when assessed on muscular repair process[12], although there are no studies testing this biological material under a mechanical perspective after injury and laser irradiation, assessing its properties. Also, no studies were found in literature analyzing and quantifying the effects of the combination of plastered immobilization and low-power laser biostimulation to the mechanical properties of a muscle submitted to injury. These factors rendered the interpretation of potential changes caused by such combination difficult.

CONCLUSION

By means of mechanical analysis, we found that muscular injuries by mechanism of impact were effective. In addition, the therapeutic intervention established by means of low-power laser irradiation applied for 8 days only without associated immobilization showed mechanical behavior curves that are similar to control group when the mechanical properties of load and stretching at maximum limit and load at proportionality limit were assessed on injured muscles.
The combination of immobilization for 24 and 72 hours with laser therapy did not improve the results for mechanical properties of the tissue. The use of each treatment modality alone was more effective.

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